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Jc674 U.S. PTO

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SIR:

Transmitted herewith for filing is the patent application (including Specification, Claims, and Abstract, 68 pages) of:

Jc678 U.S. PTO  
09/412100  
10/04/99

Inventors : Zhong-Min Wei, Hao Fan, and Jennifer L. Niggemeyer

For : HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE  
ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

*\*\*If a CONTINUING APPLICATION, please mark where appropriate and supply the requisite information below and in a preliminary amendment:*

[ ] continuation [ ] divisional [ ] Continuation-In-Part (CIP)  
of prior application Serial No. \_\_\_\_\_

Prior application information: Examiner :  
Art Unit :

Enclosed are:

- ☒ 2 sheets of informal drawings.
- ☐ Signed Combined Declaration and Power of Attorney (\_\_\_\_ pages).
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- ☐ Signed statement deleting inventor(s) named in prior application (\_\_\_\_ pages) (1.63(d)(2) and 1.33(b)).
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- ☐ Verified statement to establish small entity status (\_\_\_\_ pages) (newly signed or copy filed in prior application).

09412100

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- ☐ Information Disclosure Statement, form PTO-1449 (\_\_\_\_\_ pages) and \_\_\_\_\_ references.
- ☒ **UNSIGNED** Combined Declaration and Power of Attorney (2 pages).
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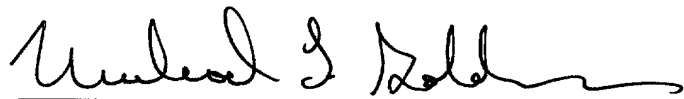
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DOCKET NO. : 21829/31 (EBC-002)

APPLICANTS : Zhong-Min Wei, Hao Fan, Jennifer L. Niggemeyer

TITLE : **HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS  
WHICH ARE ACTIVE BUT DO NOT ELICIT A  
HYPERSENSITIVE RESPONSE**

Certificate is attached to the **Patent Application including specification, claims, and abstract (68 pages), Sequence Listing (29 pages), and UNSIGNED Combined Declaration and Power of Attorney Form (2 pages)** of the above-named application.

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**TITLE:**                   **HYPERSENSITIVE RESPONSE ELICITOR  
FRAGMENTS WHICH ARE ACTIVE BUT  
DO NOT ELICIT A HYPERSENSITIVE  
RESPONSE**

**INVENTORS:**           **Zhong-Min Wei, Hao Fan, and Jennifer L.  
Niggemeyer**

**DOCKET NO.:**       **21829/31 (EBC-002)**

SECRET

## **HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE**

This application claims benefit of U.S. Provisional Patent Application  
5 Serial No. 60/103,050, filed October 5, 1998.

### **FIELD OF THE INVENTION**

The present invention relates to active fragments of a hypersensitive  
10 response elicitor which fragments do not elicit a hypersensitive response.

### **BACKGROUND OF THE INVENTION**

Interactions between bacterial pathogens and their plant hosts generally  
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular  
bacterial growth, symptom development, and disease development in the host plant;  
and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a  
particular type of incompatible interaction occurring, without progressive disease  
symptoms. During compatible interactions on host plants, bacterial populations  
20 increase dramatically and progressive symptoms occur. During incompatible  
interactions, bacterial populations do not increase, and progressive symptoms do not  
occur.

The hypersensitive response is a rapid, localized necrosis that is  
associated with the active defense of plants against many pathogens (Kiraly, Z.,  
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant  
Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed.  
Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177  
in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic  
Press, New York (1982)). The hypersensitive response elicited by bacteria is readily  
30 observed as a tissue collapse if high concentrations ( $\geq 10^7$  cells/ml) of a limited  
host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated  
into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower  
levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of  
Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

“Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf,” Phytopathology 54:474-477 (1963); Turner, et al., “The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction,” Phytopathology 64:885-890 (1974); Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., “Gene Cluster of *Pseudomonas syringae* pv. ‘phaseolicola’ Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants,” J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., “*hrp* Genes of Phytopathogenic Bacteria,” pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangel, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., “Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria,” Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. “*Pseudomonas Syringae* pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” Cell 73:1255-1266 (1993), Wei, Z.-H.,

et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al.

"PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum* *popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin<sub>Ech</sub>: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA<sup>-</sup> Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments do not elicit a hypersensitive response but are active when utilized in conjunction with plants.

5

## SUMMARY OF THE INVENTION

The present invention is directed to isolated fragments of an *Erwinia* hypersensitive response elicitor protein or polypeptide which fragments do not elicit a hypersensitive response in plants but are otherwise active when utilized in  
10 conjunction with plants. Also disclosed are isolated DNA molecules which encode such fragments.

The fragments of hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling  
15 insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects  
20 on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control  
25 insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

30



## **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

5                Figure 2 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ. ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8),  
10            N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

## **DETAILED DESCRIPTION OF THE INVENTION**

15                The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments do not  
20            elicit a hypersensitive response but have other activity in plants. Also disclosed are DNA molecules encoding such fragments as well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

25                The fragments of hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein  
30            elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

5 The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

10	Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser	1	5	10	15
	Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser	20	25	30	
	Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr	35	40	45	
15	Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu	50	55	60	
	Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser	65	70	75	80
20	Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys	85	90	95	
	Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp	100	105	110	
	Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln	115	120	125	
25	Leu	Ala	Asn	Ser	Met	Leu	Asn	Ala	Ser	Gln	Met	Thr	Gln	Gly	Asn	Met	130	135	140	
	Asn	Ala	Phe	Gly	Ser	Gly	Val	Asn	Asn	Ala	Leu	Ser	Ser	Ile	Leu	Gly	145	150	155	160
30	Asn	Gly	Leu	Gly	Gln	Ser	Met	Ser	Gly	Phe	Ser	Gln	Pro	Ser	Leu	Gly	165	170	175	
	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	180	185	190	
	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	195	200	205	
35	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	210	215	220	
	Asp	Lys	Glu	Asp	Arg	Gly	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	Asp	225	230	235	240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp  
245 250 255  
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys  
260 265 270  
5 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln  
275 280 285  
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr  
290 295 300  
10 Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
305 310 315 320  
Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
325 330 335  
Asn Ala

15 This hypersensitive response elicitor polypeptide or protein has a molecular weight of  
34 kDa, is heat stable, has a glycine content of greater than 16%, and contains  
substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor  
polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence  
20 corresponding to SEQ. ID. No. 22 as follows:

CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCTGA CACCGTTACG 60  
GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC 120  
GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG 180  
25 CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG 240  
TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG 300  
CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACCT GGCGGGAATG 360  
ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC 420  
CGATCATTA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT 480  
30 CACCGTCGGC GTCACCTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG 540  
GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA 600  
AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC 660  
TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCAGCG TGGATAAACT 720  
GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT 780  
35 GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC 840

TTTCGGCAAT GCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA 900  
TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC 960  
CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC 1020  
CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCTG CCATTCTCGG 1080  
5 CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT 1140  
GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT 1200  
GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA 1260  
CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA 1320  
TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA 1380  
10 GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG 1440  
CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA 1500  
TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC 1560  
GGCTGTCGTC GCGGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA 1620  
ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680  
15 TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTAAATCA TCGTCATCGA TCTGGTACAA 1740  
ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC 1800  
GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860  
CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG 1920  
CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980  
20 GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040  
AAAATAGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100  
GTTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T 2141

25 The hypersensitive response elicitor polypeptide or protein derived  
from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.  
No. 23 as follows:

30 Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
1 5 10 15  
Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
20 25 30

	Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Gly	Asn
			35					40					45			
	Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met
		50					55					60				
5	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Gly	Leu
	65					70					75					80
	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu
					85					90					95	
10	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr
				100					105					110		
	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro
				115				120						125		
	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser
		130					135					140				
15	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln
	145					150						155				160
	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly
					165					170					175	
20	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu
				180					185					190		
	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly
			195					200					205			
	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly
		210					215					220				
25	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu
	225					230					235					240
	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln
					245					250					255	
30	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln
				260					265					270		
	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe
			275					280					285			
	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met
		290					295					300				
35	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro
	305					310					315					320
	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser
					325					330					335	
	Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn

340 345 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
355 360 365

5 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
385 390 395 400

Gly Ala Ala

This hypersensitive response elicitor polypeptide or protein has a molecular weight of  
10 about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10  
minutes. This hypersensitive response elicitor polypeptide or protein has substantially  
no cysteine. The hypersensitive response elicitor polypeptide or protein derived from  
*Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff,  
D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the  
15 Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"  
Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA  
molecule encoding this polypeptide or protein has a nucleotide sequence  
corresponding to SEQ. ID. No. 24 as follows:

20 AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA 60  
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT 120  
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG 180  
GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAAATCAA ATGATACCGT CAATCAGCTG 240  
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG 300  
25 GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA 360  
GGACTGTCTG ACGCGCTGAA CGATATGTTA GGCGGTTTCG TGAACACGCT GGGCTCGAAA 420  
GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC 480  
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC 540  
CCGATGCAGC AGCTGCTGAA GATGTTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG 600  
30 CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC 660  
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG 720  
CTCCTTGGCA ACGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC 780  
GGTTCGTCTG TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG 840

TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTTCAGGC GCTGAATGAT 900  
ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG 960  
GCGAAGGAAA TCGGTCAAGT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC 1020  
CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAA AGCACTGAGC 1080  
5 AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC 1140  
ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC 1200  
GGTGGTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA 1260  
CTTGGCAAGC TGGGCGCGGC TTAAGCTT 1288

10

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 25 as follows:

15

ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCTCTCGC CGGGTCTGTT CCAGTCCGGG 60

GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT 120

20

CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG 180

CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT 240

25

AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT 300

CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC 360

CAGGGCGGCG GGCAGATCGG CGATAATCCT TTACTGAAAG CCATGCTGAA GCTTATTGCA 420

30

CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC 480

TCTTCCGGTA CTTCTTCATC TGGCGGTTCC CCTTTTAACG ATCTATCAGG GGGGAAGGCC 540

35

CCTTCCGGCA ACTCCCCTTC CGGCAACTAC TCTCCGTC GTACCTTCTC ACCCCCATCC 600

ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTTCTT CTCCCACCAA AGCAGCCGGG 660

GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGCGGGCAT CGGGGCCGGA 720

40

AATTCGGTGG CTTTACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC 780

GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA 840

45

GGCGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CGGTGCCAGC 900

CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC 960

AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC 1020

50

AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC 1080

AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC 1140

TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC 1200  
 5 CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC 1260  
 GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC 1320  
 GCCAACCTGA AGGTGGCTGA ATGA 1344

10

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 26 as follows:

15 Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu  
 1 5 10 15  
 Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser  
 20 20 25 30  
 Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala  
 35 40 45  
 25 Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly  
 50 55 60  
 Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly  
 65 70 75 80  
 30 Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro  
 85 90 95  
 Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu  
 100 105 110  
 35 Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp  
 115 120 125  
 Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp  
 130 135 140  
 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala  
 145 150 155 160  
 45 Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
 165 170 175  
 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
 180 185 190  
 50 Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro  
 195 200 205  
 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
 210 215 220



	Val	Thr	Asp	His	Pro	Asp	Pro	Val	Gly	Ser	Ala	Gly	Ile	Gly	Ala	Gly	
	225					230					235					240	
5	Asn	Ser	Val	Ala	Phe	Thr	Ser	Ala	Gly	Ala	Asn	Gln	Thr	Val	Leu	His	
					245					250					255		
	Asp	Thr	Ile	Thr	Val	Lys	Ala	Gly	Gln	Val	Phe	Asp	Gly	Lys	Gly	Gln	
				260					265					270			
10	Thr	Phe	Thr	Ala	Gly	Ser	Glu	Leu	Gly	Asp	Gly	Gly	Gln	Ser	Glu	Asn	
			275					280					285				
	Gln	Lys	Pro	Leu	Phe	Ile	Leu	Glu	Asp	Gly	Ala	Ser	Leu	Lys	Asn	Val	
15		290					295					300					
	Thr	Met	Gly	Asp	Asp	Gly	Ala	Asp	Gly	Ile	His	Leu	Tyr	Gly	Asp	Ala	
	305					310					315					320	
	Lys	Ile	Asp	Asn	Leu	His	Val	Thr	Asn	Val	Gly	Glu	Asp	Ala	Ile	Thr	
20					325					330					335		
	Val	Lys	Pro	Asn	Ser	Ala	Gly	Lys	Lys	Ser	His	Val	Glu	Ile	Thr	Asn	
					340				345					350			
25	Ser	Ser	Phe	Glu	His	Ala	Ser	Asp	Lys	Ile	Leu	Gln	Leu	Asn	Ala	Asp	
			355					360					365				
	Thr	Asn	Leu	Ser	Val	Asp	Asn	Val	Lys	Ala	Lys	Asp	Phe	Gly	Thr	Phe	
30							375					380					
	Val	Arg	Thr	Asn	Gly	Gly	Gln	Gln	Gly	Asn	Trp	Asp	Leu	Asn	Leu	Ser	
	385					390					395					400	
	His	Ile	Ser	Ala	Glu	Asp	Gly	Lys	Phe	Ser	Phe	Val	Lys	Ser	Asp	Ser	
35					405					410					415		
	Glu	Gly	Leu	Asn	Val	Asn	Thr	Ser	Asp	Ile	Ser	Leu	Gly	Asp	Val	Glu	
				420					425					430			
40	Asn	His	Tyr	Lys	Val	Pro	Met	Ser	Ala	Asn	Leu	Lys	Val	Ala	Glu		
			435					440					445				

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663 which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 27 as follows:

	ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAAC	60
	CCTGTGGGGC ATGGTGTGTC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC	120
5	GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA	180
	TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG	240
10	GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC	300
	CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT	360
	GAGGCGGCCG CGCCAGATGC GGCGCGTTTG ACCCGTTTCG GCGGCGTCAA ACGCCGCAAT	420
15	ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA	480
	ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC	540
20	AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCATCGCC TGCAGCATTC ACCGCCGAC	600
	ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA	660
	ACGGCCCCAG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
25	CTGCATCAAC AGCGGTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC	780
	GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAACTGA CTGCGGTTGC GGAAAGCGTC	840
30	CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
	GGAGCCGGGG TAACCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
	GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CCCCCAGCAC	1020
35	TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
	CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
40	GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200
	AGCGGCAAGA TCTCGTGGG GAGCGGTACG CAAAGTCACA ACAAACAAT GCTAAGCCAA	1260
	CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTGTC AGCATCCTGC TGGCGCAGCG	1320
45	CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG	1380
	CTGGGCGTAT GGCAATCTGC GGATAAAGAT ACCCAGGCC AGCTGTCTCG CCAGGCAGAC	1440
50	GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC	1500
	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCGGTTG ATCAGCGGGG GCAGGTGGCG	1560
	ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620
55	CCGAGAGGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC	1680
	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTTGTGGCC	1740
60	GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG	1800
	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT	1860
	TCTGGATTTT TCCATGACGA CCACGCCAG CTTAATGCGC TGGTGAAAAA TAACTTCAGG	1920
65	CAGCAGCATG CCTGCCCGTT GGGTAACGAT CATCAGTTTC ACCCGGCTG GAACCTGACT	1980

	GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG	2040
5	ATTCTTGATA TGGGGCATTT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT	2100
	GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAAGGC	2160
	CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCTGAA TATTAATCAG	2220
10	AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT	2280
	AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG	2340
15	GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC	2400
	CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAACCTCTCAG CCGCGAAGGT	2460
	ATCAGCGGCG AACTGAAAGA CATTCATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC	2520
20	CACGAGGGAG AGGTGTTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC	2580
	AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC	2640
25	CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC	2700
	GGCTGGCAGC CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA	2760
	CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG	2820
30	TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC	2880
	AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG	2940
35	CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG	3000
	AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAACAAC TGGATGCGCA TAACGTTCGT	3060
	CATAACGCGC CACAGCCAGA TTTGCAGAGC AAACCTGGAAA CTCTGGATTT AGGCGAACAT	3120
40	GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGACG AACTGGAGCA GAGTGCAACC	3180
	CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT	3240
45	AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT	3300
	GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGAGAG	3360
	AGTAAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG	3420
50	AAGGGCGAGA TCCCCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAAA	3480
	TCGCGTTTAA TTTTAGATAC CGTGACCATC GGTGAACTGC ATGAACTGGC CGATAAGGCG	3540
55	AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC	3600
	GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC	3660
	TTCACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC	3720
60	TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC	3780
	AGTGCGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAAGT	3840
65	ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCTTAGC	3900

AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC 3960  
 CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG 4020  
 5 AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA 4080  
 ACCAGTGCAG GTAACGCCAG TGACTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG 4140  
 10 CGTATCGGCG CTGCTGTGAG TGGCACCCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT 4200  
 AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC 4260  
 CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG 4320  
 15 TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC 4380  
 GGCAGTAAAC CAAATGGTGT CACTGCCCCTG GTTCTGCGG GGCTAAGTGC ATCGGCAAAAC 4440  
 20 CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG 4500  
 GCCAGCAATA ACCGCCCAAC CTTCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT 4560  
 GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAAC CGGTCGGGAT CTTCCCGGCA 4620  
 25 TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC 4680  
 AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC 4740  
 30 ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG 4800  
 TTAGATGACG CTAAGCCCGC TGAACAACG CATATTTTAC AGCAGCATTT CAGTGCAAAA 4860  
 GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAAT GGTGATACGT 4920  
 35 CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC 4980  
 AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTCGAGC TGCTACACAA ACATTTTCGAT 5040  
 40 GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA 5100  
 CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCGATG 5160  
 GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT 5220  
 45 CGTGAAGAAG TGGGAGTACT TTCCAGGAT CGTAACAACT TCGTGTTAA ATCGGTCAGC 5280  
 GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG 5340  
 AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC 5400  
 50 CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG 5460  
 CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA 5517

55 This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated  
 DNA molecule of the present invention encodes a protein or polypeptide which elicits  
 a plant pathogen's hypersensitive response having an amino acid sequence of SEQ.  
 ID. No. 28 as follows:

60

	Met	Glu	Leu	Lys	Ser	Leu	Gly	Thr	Glu	His	Lys	Ala	Ala	Val	His	Thr
	1				5				10					15		
5	Ala	Ala	His	Asn	Pro	Val	Gly	His	Gly	Val	Ala	Leu	Gln	Gln	Gly	Ser
				20					25					30		
	Ser	Ser	Ser	Ser	Pro	Gln	Asn	Ala	Ala	Ala	Ser	Leu	Ala	Ala	Glu	Gly
			35					40					45			
10	Lys	Asn	Arg	Gly	Lys	Met	Pro	Arg	Ile	His	Gln	Pro	Ser	Thr	Ala	Ala
		50					55					60				
	Asp	Gly	Ile	Ser	Ala	Ala	His	Gln	Gln	Lys	Lys	Ser	Phe	Ser	Leu	Arg
15	65					70					75					80
	Gly	Cys	Leu	Gly	Thr	Lys	Lys	Phe	Ser	Arg	Ser	Ala	Pro	Gln	Gly	Gln
					85					90					95	
20	Pro	Gly	Thr	Thr	His	Ser	Lys	Gly	Ala	Thr	Leu	Arg	Asp	Leu	Leu	Ala
					100				105					110		
	Arg	Asp	Asp	Gly	Glu	Thr	Gln	His	Glu	Ala	Ala	Ala	Pro	Asp	Ala	Ala
			115					120					125			
25	Arg	Leu	Thr	Arg	Ser	Gly	Gly	Val	Lys	Arg	Arg	Asn	Met	Asp	Asp	Met
		130					135					140				
	Ala	Gly	Arg	Pro	Met	Val	Lys	Gly	Gly	Ser	Gly	Glu	Asp	Lys	Val	Pro
30	145					150					155					160
	Thr	Gln	Gln	Lys	Arg	His	Gln	Leu	Asn	Asn	Phe	Gly	Gln	Met	Arg	Gln
					165					170					175	
35	Thr	Met	Leu	Ser	Lys	Met	Ala	His	Pro	Ala	Ser	Ala	Asn	Ala	Gly	Asp
				180					185					190		
	Arg	Leu	Gln	His	Ser	Pro	Pro	His	Ile	Pro	Gly	Ser	His	His	Glu	Ile
			195					200					205			
40	Lys	Glu	Glu	Pro	Val	Gly	Ser	Thr	Ser	Lys	Ala	Thr	Thr	Ala	His	Ala
		210					215					220				
	Asp	Arg	Val	Glu	Ile	Ala	Gln	Glu	Asp	Asp	Asp	Ser	Glu	Phe	Gln	Gln
45	225					230					235					240
	Leu	His	Gln	Gln	Arg	Leu	Ala	Arg	Glu	Arg	Glu	Asn	Pro	Pro	Gln	Pro
					245					250					255	
50	Pro	Lys	Leu	Gly	Val	Ala	Thr	Pro	Ile	Ser	Ala	Arg	Phe	Gln	Pro	Lys
				260					265					270		
	Leu	Thr	Ala	Val	Ala	Glu	Ser	Val	Leu	Glu	Gly	Thr	Asp	Thr	Thr	Gln
			275					280					285			
55	Ser	Pro	Leu	Lys	Pro	Gln	Ser	Met	Leu	Lys	Gly	Ser	Gly	Ala	Gly	Val
		290					295					300				
	Thr	Pro	Leu	Ala	Val	Thr	Leu	Asp	Lys	Gly	Lys	Leu	Gln	Leu	Ala	Pro
60	305					310					315					320
	Asp	Asn	Pro	Pro	Ala	Leu	Asn									

		His	Leu	Leu	Leu	Asp	Asn	Lys	Gly	His	Leu	Phe	Asp	Ile	Lys	Ser	Thr
			355						360					365			
5		Ala	Thr	Ser	Tyr	Ser	Val	Leu	His	Asn	Ser	His	Pro	Gly	Glu	Ile	Lys
			370					375					380				
		Gly	Lys	Leu	Ala	Gln	Ala	Gly	Thr	Gly	Ser	Val	Ser	Val	Asp	Gly	Lys
10			385				390					395				400	
		Ser	Gly	Lys	Ile	Ser	Leu	Gly	Ser	Gly	Thr	Gln	Ser	His	Asn	Lys	Thr
					405						410					415	
		Met	Leu	Ser	Gln	Pro	Gly	Glu	Ala	His	Arg	Ser	Leu	Leu	Thr	Gly	Ile
15					420					425					430		
		Trp	Gln	His	Pro	Ala	Gly	Ala	Ala	Arg	Pro	Gln	Gly	Glu	Ser	Ile	Arg
			435						440					445			
20		Leu	His	Asp	Asp	Lys	Ile	His	Ile	Leu	His	Pro	Glu	Leu	Gly	Val	Trp
			450					455					460				
		Gln	Ser	Ala	Asp	Lys	Asp	Thr	His	Ser	Gln	Leu	Ser	Arg	Gln	Ala	Asp
25			465				470					475					480
		Gly	Lys	Leu	Tyr	Ala	Leu	Lys	Asp	Asn	Arg	Thr	Leu	Gln	Asn	Leu	Ser
					485						490					495	
		Asp	Asn	Lys	Ser	Ser	Glu	Lys	Leu	Val	Asp	Lys	Ile	Lys	Ser	Tyr	Ser
30					500					505					510		
		Val	Asp	Gln	Arg	Gly	Gln	Val	Ala	Ile	Leu	Thr	Asp	Thr	Pro	Gly	Arg
			515						520					525			
35		His	Lys	Met	Ser	Ile	Met	Pro	Ser	Leu	Asp	Ala	Ser	Pro	Glu	Ser	His
			530					535					540				
		Ile	Ser	Leu	Ser	Leu	His	Phe	Ala	Asp	Ala	His	Gln	Gly	Leu	Leu	His
40			545				550					555					560
		Gly	Lys	Ser	Glu	Leu	Glu	Ala	Gln	Ser	Val	Ala	Ile	Ser	His	Gly	Arg
					565						570					575	
		Leu	Val	Val	Ala	Asp	Ser	Glu	Gly	Lys	Leu	Phe	Ser	Ala	Ala	Ile	Pro
45					580					585					590		
		Lys	Gln	Gly	Asp	Gly	Asn	Glu	Leu	Lys	Met	Lys	Ala	Met	Pro	Gln	His
			595					600						605			
50		Ala	Leu	Asp	Glu	His	Phe	Gly	His	Asp	His	Gln	Ile	Ser	Gly	Phe	Phe
			610					615					620				
		His	Asp	Asp	His	Gly	Gln	Leu	Asn	Ala	Leu	Val	Lys	Asn	Asn	Phe	Arg
55			625				630					635					640
		Gln	Gln	His	Ala	Cys	Pro	Leu	Gly	Asn	Asp	His	Gln	Phe	His	Pro	Gly
					645						650					655	
		Trp	Asn	Leu	Thr	Asp	Ala	Leu	Val	Ile	Asp						

	Ser	Leu	Ala	Leu	Gln	Glu	Gly	Lys	Leu	His	Tyr	Phe	Asp	Gln	Leu	Thr	
	690						695					700					
5	Lys	Gly	Trp	Thr	Gly	Ala	Glu	Ser	Asp	Cys	Lys	Gln	Leu	Lys	Lys	Gly	
	705					710					715					720	
	Leu	Asp	Gly	Ala	Ala	Tyr	Leu	Leu	Lys	Asp	Gly	Glu	Val	Lys	Arg	Leu	
					725					730					735		
10	Asn	Ile	Asn	Gln	Ser	Thr	Ser	Ser	Ile	Lys	His	Gly	Thr	Glu	Asn	Val	
				740					745					750			
	Phe	Ser	Leu	Pro	His	Val	Arg	Asn	Lys	Pro	Glu	Pro	Gly	Asp	Ala	Leu	
15				755				760					765				
	Gln	Gly	Leu	Asn	Lys	Asp	Asp	Lys	Ala	Gln	Ala	Met	Ala	Val	Ile	Gly	
		770					775					780					
20	Val	Asn	Lys	Tyr	Leu	Ala	Leu	Thr	Glu	Lys	Gly	Asp	Ile	Arg	Ser	Phe	
	785				790						795					800	
	Gln	Ile	Lys	Pro	Gly	Thr	Gln	Gln	Leu	Glu	Arg	Pro	Ala	Gln	Thr	Leu	
				805					810					815			
25	Ser	Arg	Glu	Gly	Ile	Ser	Gly	Glu	Leu	Lys	Asp	Ile	His	Val	Asp	His	
				820				825						830			
	Lys	Gln	Asn	Leu	Tyr	Ala	Leu	Thr	His	Glu	Gly	Glu	Val	Phe	His	Gln	
30				835				840					845				
	Pro	Arg	Glu	Ala	Trp	Gln	Asn	Gly	Ala	Glu	Ser	Ser	Ser	Trp	His	Lys	
		850				855				860							
35	Leu	Ala	Leu	Pro	Gln	Ser	Glu	Ser	Lys	Leu	Lys	Ser	Leu	Asp	Met	Ser	
	865				870					875						880	
	His	Glu	His	Lys	Pro	Ile	Ala	Thr	Phe	Glu	Asp	Gly	Ser	Gln	His	Gln	
				885				890						895			
40	Leu	Lys	Ala	Gly	Gly	Trp	His	Ala	Tyr	Ala	Ala	Pro	Glu	Arg	Gly	Pro	
				900				905					910				
	Leu	Ala	Val	Gly	Thr	Ser	Gly	Ser	Gln	Thr	Val	Phe	Asn	Arg	Leu	Met	
45				915			920					925					
	Gln	Gly	Val	Lys	Gly	Lys	Val	Ile	Pro	Gly	Ser	Gly	Leu	Thr	Val	Lys	
		930				935				940							
50	Leu	Ser	Ala	Gln	Thr	Gly	Gly	Met	Thr	Gly	Ala	Glu	Gly	Arg	Lys	Val	
	945				950				955							960	
	Ser	Ser	Lys	Phe	Ser	Glu	Arg	Ile	Arg	Ala	Tyr	Ala	Phe	Asn	Pro	Thr	
				965				970					975				
55	Met	Ser	Thr	Pro	Arg	Pro	Ile	Lys	Asn	Ala	Ala	Tyr	Ala	Thr	Gln	His	
				980				985					990				
	Gly	Trp	Gln	Gly	Arg	Glu	Gly	Leu	Lys	Pro	Leu	Tyr	Glu	Met	Gln	Gly	
60				995			1000					1005					
	Ala	Leu	Ile	Lys	Gln	Leu	Asp	Ala	His	Asn	Val	Arg	His	Asn	Ala	Pro	
		1010				1015			1020								
65	Gln	Pro	Asp	Leu	Gln	Ser	Lys	Leu	Glu	Thr	Leu	Asp	Leu	Gly	Glu	His	
	1025				1030				1035				1040				

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	Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu	
	1045	1050 1055
5	Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val	
	1060	1065 1070
	Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly	
10	1075	1080 1085
	Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu	
	1090	1095 1100
15	Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu	
	1105	1110 1115 1120
	Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp	
	1125	1130 1135
20	Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro	
	1140	1145 1150
	Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val	
25	1155	1160 1165
	Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser	
	1170	1175 1180
30	Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe	
	1185	1190 1195 1200
	Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr	
	1205	1210 1215
35	Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp	
	1220	1225 1230
	Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val	
40	1235	1240 1245
	Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu	
	1250	1255 1260
45	Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser	
	1265	1270 1275 1280
	Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val	
	1285	1290 1295
50	Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly	
	1300	1305 1310
	Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly	
55	1315	1320 1325
	Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile	
	1330	1335 1340
60	Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys	
	1345	1350 1355 1360
	Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile	
	1365	1370 1375



	Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly	1380	1385	1390
5	Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro	1395	1400	1405
	Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu	1410	1415	1420
10	Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr	1425	1430	1435
	Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn	1445	1450	1455
15	Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser	1460	1465	1470
20	Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg	1475	1480	1485
	Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn	1490	1495	1500
25	Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala	1505	1510	1515
	Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly	1525	1530	1535
30	Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu	1540	1545	1550
35	Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu	1555	1560	1565
	Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys	1570	1575	1580
40	His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu	1585	1590	1595
	Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His	1605	1610	1615
45	Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg	1620	1625	1630
50	Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser	1635	1640	1645
	Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser	1650	1655	1660
55	Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp	1665	1670	1675
	Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn	1685	1690	1695
60	Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro	1700	1705	1710
65	Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu	1715	1720	1725

5           Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val  
               1730                       1735                       1740  
           Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser  
               1745                       1750                       1755                       1760  
           Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu  
 10                                       1765                       1770                       1775  
           Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile  
                                       1780                       1785                       1790  
 15           Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg  
                                       1795                       1800                       1805  
           Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser  
                                       1810                       1815                       1820  
 20           Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser  
               1825                       1830                       1835

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

25           The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 29 as follows:

ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAG           60  
 30   ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG           120  
      GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG           180  
 35   GCTGACCCAC AAACCTCAAT AACCCCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG           240  
      GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT           300  
      CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC           360  
 40   GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA           420

45           This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 30 as follows:

          Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser  
               1                       5                       10                       15  
 50           Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu  
                                       20                       25                       30  
           Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His  
                                       35                       40                       45  
 55           Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln  
               50                       55                       60

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The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.

25

30

35

40

Met 1	Gln	Ser	Leu	Ser 5	Leu	Asn	Ser	Ser	Ser 10	Leu	Gln	Thr	Pro	Ala 15	Met
Ala	Leu	Val	Leu 20	Val	Arg	Pro	Glu	Ala 25	Glu	Thr	Thr	Gly	Ser 30	Thr	Ser
Ser	Lys	Ala 35	Leu	Gln	Glu	Val	Val 40	Val	Lys	Leu	Ala	Glu 45	Glu	Leu	Met
Arg 50	Asn	Gly	Gln	Leu	Asp	Asp 55	Ser	Ser	Pro	Leu	Gly 60	Lys	Leu	Leu	Ala
Lys 65	Ser	Met	Ala	Ala	Asp 70	Gly	Lys	Ala	Gly	Gly 75	Gly	Ile	Glu	Asp	Val 80
Ile	Ala	Ala	Leu	Asp 85	Lys	Leu	Ile	His	Glu 90	Lys	Leu	Gly	Asp	Asn 95	Phe
Gly	Ala	Ser	Ala 100	Asp	Ser	Ala	Ser	Gly 105	Thr	Gly	Gln	Gln	Asp 110	Leu	Met
Thr	Gln	Val 115	Leu	Asn	Gly	Leu	Ala 120	Lys	Ser	Met	Leu	Asp 125	Asp	Leu	Leu
Thr 130	Lys	Gln	Asp	Gly	Gly	Thr 135	Ser	Phe	Ser	Glu	Asp 140	Asp	Met	Pro	Met
Leu 145	Asn	Lys	Ile	Ala	Gln 150	Phe	Met	Asp	Asp	Asn 155	Pro	Ala	Gln	Phe	Pro 160
Lys	Pro	Asp	Ser	Gly 165	Ser	Trp	Val	Asn	Glu 170	Leu	Lys	Glu	Asp	Asn 175	Phe
Leu	Asp	Gly	Asp 180	Glu	Thr	Ala	Ala	Phe 185	Arg	Ser	Ala	Leu	Asp 190	Ile	Ile

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
 195 200 205  
 Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
 210 215 220  
 5 Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
 225 230 235 240  
 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
 245 250 255  
 10 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
 260 265 270  
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
 275 280 285  
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290 295 300  
 15 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305 310 315 320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325 330 335  
 20 Asn Gln Ala Ala Ala  
 340

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

25 Further information about the hypersensitive response elicitor derived from  
*Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer,  
 "Pseudomonas syringae pv. syringae Harpin<sub>PSS</sub>: A Protein that is Secreted via the Hrp  
 Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266  
 (1993), which is hereby incorporated by reference. The DNA molecule encoding the  
 30 hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide  
 sequence corresponding to SEQ. ID. No. 32 as follows:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTCTG 60  
 GTACGTCTCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120  
 35 GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180  
 AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240  
 ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300

GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360  
AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420  
GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480  
AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540  
5 GAAACGGCTG CGTTCCGTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600  
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660  
AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720  
GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780  
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCC GCAGAC CGGTACGTCG 840  
10 GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900  
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960  
GCGCAAATCG CCACCTTGCT GGTGAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020  
GCCTGA 1026

15 Another potentially suitable hypersensitive response elicitor from  
*Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817,  
which is hereby incorporated by reference. The protein has a nucleotide sequence of  
SEQ. ID. No. 33 as follows:

20 TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG 60  
CTGAGTGCGC AGATTTCTGT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAAGG 120  
CCTCTGAGTG CGGTGCGGAG CAATACCAGT CTTCTGCTG GCGTGTGCAC ACTGAGTCGC 180  
25 AGGCATAGGC ATTTGAGTTC CTTGCGTTGG TTGGGCATAT AAAAAAGGA ACTTTTAAAA 240  
ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG 300  
30 AGCAAGCTCA ACCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC 360  
TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCAC TTAGCGAGGT AACGCAGCAT 420  
GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCAG CCACTCGATT TTTCGGCGCT 480  
35 AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA 540  
CCCAGTGCA CTGTTGTTTC GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCGA 600  
40 CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC 660  
TAAATTGATC AGTGCATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA 720  
GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT 780

CGGTACACCG TCGGCCGATA GCGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG 840  
 5 CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG 900  
 TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC 960  
 CACTGCAACA GCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA 1020  
 10 CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTCTTA CCGAGCAAGC 1080  
 CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG 1140  
 15 CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA 1200  
 GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA 1260  
 CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT 1320  
 20 GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAA GCGAGGGAG GCGCAGCGGT 1380  
 CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT 1440  
 25 CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTTCG GCACGATGGT 1500  
 TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC 1560  
 TAACCACGGC AAGTTCGCCC TGGTAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG 1620  
 30 CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680  
 CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAGGG GGTGGACTC 1729

35 This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 34 as follows:

40 Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu  
 1 5 10 15  
 Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly  
 20 25 30  
 45 Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly  
 35 40 45  
 50 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val  
 50 55 60  
 Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile  
 65 70 75 80  
 55 Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr  
 85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln  
100 105 110

5 Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser  
115 120 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr  
130 135 140

10 Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly  
145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly  
165 170 175

15 Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr  
180 185 190

20 Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr  
195 200 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile  
210 215 220

25 Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp  
225 230 235 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp  
245 250 255

30 Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr  
260 265 270

35 Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val  
275 280 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln  
290 295 300

40 Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala  
305 310 315 320

Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp  
325 330 335

45 Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe  
340 345 350

50 Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln  
355 360 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly  
370 375 380

55 Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr  
385 390 395 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln  
405 410 415

Ala Ser Thr Gln His Thr Glu Leu  
420

5

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived  
from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.

10 ID. No. 35 as follows:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln  
1 5 10 15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser  
15 20 25 30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile  
35 40 45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly  
50 55 60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala  
20 65 70 75 80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser  
85 90 95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met  
25 100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
30 145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
35 180 185 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
210 215 220



Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
245 250 255

5 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
275 280 285

10 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
325 330 335

15 Gln Ser Thr Ser Thr Gln Pro Met  
340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.  
ID. No. 36 as follows:

ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC 60

20 AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC 120

GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC 180

GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC 240

AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC 300

GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA 360

25 GACCTGGTGA AGCTGCTGAA GCGGCGCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG 420

GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC 480

GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC 540

GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT 600

GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC 660

30 GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC 720

CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGACG 780

ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC 840

GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT 900

GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960  
 GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020  
 ACGCAGCCGA TGTA 1035

5 Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted  
 10 via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 37 as follows:

15 Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala  
 1 5 10 15  
 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr  
 20 25

20 This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other  
 25 *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 38 as follows:

30 Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln  
 1 5 10 15  
 Leu Leu Ala Met  
 20

35 Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves,” MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., “Harpin is Not

- 5 Necessary for the Pathogenicity of *Erwinia stewartii* on Maize,” 8th Int’l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., “Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize,” Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from

- 10 *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., “Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens,” Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., “Structure and
- 15 Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco,” Eur. J. Biochem., 183:555-63 (1989), Ricci et al., “Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*,” Plant Path. 41:298-307 (1992), Baillreul et al, “A New Elicitor of the Hypersensitive Response in Tobacco: A
- 20 Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance,” Plant J., 8(4):551-60 (1995), and Bonnet et al., “Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants,” Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

- 25 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

- 30 The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture

supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia amylovora* hypersensitive response elicitor. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid

sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of a useful fragment of a hypersensitive response elicitor which fragment does not itself elicit a hypersensitive response is the protein fragment containing amino acids 190 to 294 of the amino acid sequence (SEQ. ID. No. 31) for the *Pseudomonas syringae* pv. *syringae* hypersensitive response elicitor.

This fragment is useful in imparting disease resistance and enhancing plant growth.

Yet another example of a useful fragment of a hypersensitive response elicitor is the peptide having an amino acid sequence corresponding to SEQ. ID. No. 39. This peptide is derived from the hypersensitive response eliciting glycoprotein of *Phytophthora megasperma* and enhances plant growth.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The fragment of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the fragment of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the fragment is separated by centrifugation. The supernatant fraction containing the fragment is subjected to gel filtration in an

appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A

Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their “strength” (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the  $P_R$  and  $P_L$  promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in “strength” as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various “strong” transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5’ to the initiation codon (“ATG”) to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.



Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor polypeptide or protein which does not elicit a hypersensitive response in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant seed cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention:

- 5 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

- 10 With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds  
15 germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed  
20 germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

- Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present  
25 invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from  
30 releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the fragment of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with the fragment of the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

5                   A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains  
10 greater than 500 nM of the fragment.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

15                   Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

20                   In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a fragment of a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art.

25                   The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby  
30 incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic

transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy

root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition,  
5 assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science,  
10 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad.  
15 Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form  
20 plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and  
25 repeatable.  
30

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves  
5 can be cultivated in accordance with conventional procedure with the presence of the gene encoding the fragment of the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant.

Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using  
10 conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the  
15 polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a fragment of a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including  
20 a fragment of a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the fragment of a  
25 hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

## EXAMPLES

30

### **Example 1 - Bacterial Strains and Plasmids**

*Escherichia coli* strains used in the following examples include DH5 $\alpha$  and BL21(DE3) purchased from Gibco BRL (Grand Island, N.Y.) and Stratagene



(La Jolla, CA), respectively. The pET28(b) vector was purchased from Novagen (Madison, WI). Eco DH5 $\alpha$ /2139 contained the complete *hrpN* gene. The 2139 construct was produced by D. Bauer at Cornell University. The *hrpN* gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then  
5 purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated *hrpN* clones. These clones were subsequently inserted into the (His)<sub>6</sub> vector pET28(b) which contained a Kan<sup>r</sup> gene for selection of transformants.

### **Example 2 - DNA Manipulation**

10 Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN) or Gibco BRL. T4 DNA ligase, Calf Intestinal Alkaline Phosphatase (CIAP), and PCR Supermix<sup>TM</sup> were obtained from Gibco BRL. The QIAprep Spin Miniprep Kit, the Qiagen Plasmid Mini Kit, and the QIAquick PCR  
15 Purification Kit were purchased from Qiagen (Hilden, Germany). The PCR primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). The oligopeptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX). All DNA manipulations such as plasmid isolation, restriction enzyme digestion, DNA ligation, and PCR were performed according to standard techniques (Sambrook, et al., Laboratory Manual,  
20 Second Edition, Cold Spring Harbor Laboratory Press (1989)) or protocols provided by the manufacturer.

### **Example 3 - Fragmentation of *hrpN* Gene**

25 A series of N-terminal and C-terminal truncated *hrpN* genes and internal fragments were generated via PCR (Fig. 1). The full length *hrpN* gene was used as the DNA template and 3' and 5' primers were designed for each truncated clone (Fig. 2). The 3' primers contained an NdeI enzyme cutting site which contained the start codon ATG (methionine) and the 5' primers contained the stop codon TAA  
30 and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was carried out in 0.5 ml tubes in a GeneAmp<sup>TM</sup> 9700 (Perkin-Elmer, Foster City, CA). 45  $\mu$ l of Supermix<sup>TM</sup> (Life Technology, Gaithersburg, MD) were mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and deionized

H<sub>2</sub>O to a final volume of 50 µl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex, San Diego, CA).

Amplified DNA was purified with the QIAquick PCR purification kit, digested with

5 Nde I and Hind III at 37°C for 5 hours, extracted once with

phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 µg of

pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting

from incomplete single enzyme digestion. Digested vector DNA was purified with

10 the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 µl mixture containing ca. 200 ng of digested

pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 µl of ligation solution were added to 100 µl of DH5α competent cells in a 15 ml Falcon

tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9

15 ml SOC solution or 0.45 ml LB media were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 µl of transformed cells were placed onto LB agar with

30 µg/ml of kanamycin and incubated at 37°C overnight. Single colonies were

transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit (QIAGEN, Hilden,

20 Germany). The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids

with the desired DNA sequence were transferred into the BL21 strain using the

standard chemical transformation method as indicated above. A clone containing the

full length harpin protein in the pET28(b) vector was generated as a positive control,

25 and a clone with only the pET28(b) vector was generated as a negative control.

#### **Example 4 - Expression of Hypersensitive Response Elicitor Truncated Proteins**

*Escherichia coli* BL21(DE3) strains containing the hrpN clones were

30 grown in Luria broth medium (5g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5 g/L NaCl, and 1 mM NaOH) containing 30 µg/ml of kanamycin at 37°C overnight. The

bacteria were then inoculated into 100 volumes of the same medium and grown at

37°C to an OD<sub>620</sub> of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD<sub>620</sub> of ca. 0.3 or 0.6-0.8. One millimolar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH<sub>2</sub>PO<sub>4</sub>, and 0.72 K<sub>2</sub>HPO<sub>4</sub>), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 1).

Table 1: Expression of hypersensitive response elicitor truncated proteins

Fragment	amino acids (SEQ. ID. No. 23)	Growth medium	Induction O.D.	Expression temp.	Harvest time
1 (+ control)	1-403	LB	ca. 0.3 or 0.6-0.8	19°C or 25°C	16-18 hr
2 (+ control)	-	LB and TB	ca. 0.3 or 0.6-0.8	19 C and 37 C	16-18 hr
3	105-403	LB	0.6-0.8	19°C	16-18 hr
4	169-403	TB	ca. 0.3	19°C	16-18 hr
5	210-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
6	257-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
7	343-403	LB	ca. 0.3	19°C	5 hr
8	1-75	TB	ca. 0.3	37°C	16-18 hr
9	1-104	TB	ca. 0.3	37°C	16-18 hr
10	1-168	TB	ca. 0.3	37°C	16-18 hr
11	1-266	LB	ca. 0.3	37°C	4 hr
12	1-342	LB	0.6-0.8	19°C	16-18 hr
13	76-209	LB	ca. 0.3	37°C	5 hr
14	76-168	TB or LB	ca. 0.3	37°C	3 hr or 16-18 hr
15	105-209	M9ZB	ca. 0.3	37°C	3 hr
16	169-209	no expression			
17	105-168	LB	ca. 0.3	37°C	3-5 hr
18	99-209	LB	ca. 0.3	37°C	3 hr
19	137-204	LB	ca. 0.3	37°C	3 hr
20	137-180	LB	ca. 0.3	37°C	16-18 hr.
21	105-180	LB	ca. 0.3	37°C	3 hr
22	150-209	no expression			
23	150-180	no expression			

#### Example 5 - Small Scale Purification of Hypersensitive Response Elicitor Truncated Proteins (Verification of Expression)

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell

suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant saved. A 50 µl aliquot of a 50% slurry of an equilibrated (His)<sub>6</sub>-  
5 binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 µl of urea elution buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3).  
10 The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

#### **Example 6 - Induction of HR in Tobacco**

A 1.5 ml aliquot from the 50 ml cultures grown for small scale  
15 purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf  
20 panes and infiltrating the bacterial lysate into the intercellular leaf space using a syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins  
25 for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

#### **Example 7 - Large Scale Native Purification of Hypersensitive Response Elicitor Truncated Proteins for Comprehensive Biological Activity Assays**

30

Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture, the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5

mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, incubated at 30°C for 15 minutes, sonicated for two minutes, centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4 ml aliquot of a 50% slurry of an equilibrated (His)<sub>6</sub>-binding nickel agarose resin was added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

**Example 8 - Large Scale Urea Purification of Hypersensitive Response Elicitor Truncated Proteins For Comprehensive Biological Activity Assay**

The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.

**Example 9 – Induction of Growth Enhancement (GE)**

Sixty tomato (*Lycopersicon spp.* cv. Marglobe) seeds were soaked  
5 overnight in 10 and 20 µg/ml of the truncated protein diluted with 5mM potassium  
phosphate buffer, pH 6.8. The next morning, the sixty seeds were sown in three pots  
and 12-15 days later and again 18-20 days later the heights of the 10 tallest tomato  
plants per pot were measured and compared with the heights of the control plants  
treated only with phosphate buffer. Analyses were done on the heights to determine if  
10 there was a significant difference in the height of the plants treated with the truncated  
proteins compared with the buffer control, and thereby determine whether the proteins  
induced growth enhancement.

**Example 10 – Induction of Systemic Acquired Resistance (SAR)**

15 Three tobacco (*Nicotiana tabacum* cv. Xanthi) plants with 8-12 leaves  
(ca. 75 day old plants) were used in the assay. One leaf of the tobacco plants was  
covered up and the rest of the leaves were sprayed with ca. 50 ml of a 20 µg/ml  
solution of the truncated proteins diluted with 5mM potassium phosphate buffer. Five  
20 to seven days later two leaves (the unsprayed leaf and the sprayed leaf opposite and  
just above the unsprayed leaf) were inoculated with 20 µl of a 1.8 µg/ml solution of  
TMV along with a pinch of diatomaceous earth by rubbing the mixture along the top  
surface of the leaves. The TMV entered the plants through tiny lesions made by the  
diatomaceous earth. Ca. 3-4 days post TMV inoculation, the number of TMV lesions  
25 was counted on both leaves compared with the number of lesions on the negative  
control buffer treated leaves. Analyses were done to determine the efficacy of  
reducing the number of TMV lesions by the protein fragments compared to the buffer  
control. Percentage of efficacy was calculated as: Reduction in TMV lesions (%  
efficacy) =  $100 \times (1 - \text{mean \# of lesions on treated leaves} / \text{mean \# of lesions on buffer}$   
30  $\text{control leaves})$ .

**Example 11 - Expression of Hypersensitive Response Elicitor Truncated Proteins**

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 2).

5

Table 2

Expression and HR activity of hypersensitive response elicitor truncated proteins  
(small scale screening)

Fragment #	Amino Acids (SEQ. ID. No. 23)	Expression	HR activity
1(+control)	1-403	+	+
2(- control)	-	background protein only	-
3	105-403	+	+
4	169-403	+	-
5	210-403	+	-
6	267-403	+	-
7	343-403	+/-	-
8	1-75	+	-
9	1-104	+	+/-
10	1-168	+	+
11	1-266	+	+
12	1-342	+	+
13	76-209	+	+
14	76-168	+	-
15	105-209	+	+
16	169-209	-	-
17	105-168	+	-
18	99-209	+	+
19	137-204	+	+
20	137-180	+	+
21	105-180	+	+
22	150-209	-	-
23	150-180	-	-

10

All of the cloned fragment proteins were expressed at varying levels except for three small fragments (amino acids 169-209, 150-209, and 150-180). Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel

15

electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and negative control proteins, consisting of

the full length hypersensitive response elicitor protein and only background proteins, respectively, were tested for expression and HR activity as well.

The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 3).

5

Table 3

Expression level and HR titer of hypersensitive response elicitor truncated proteins (large sale purification)

10

Fragment #	Amino acids (SEQ. ID. No. 23)	Expression	HR titer
1(+ control)	1-403	3.7 mg/ml	5-7 µg/ml
2 (- control)	-	-	1:2 dilution
4	169-403	2 mg/ml	-
5	210-403	5 mg/ml	-
6	267-403	4 mg/ml	-
7	343-402	200µg/ml	-
8	1-75	50µg/ml	-
9	1-104	50µg/ml	3 µg/ml (1:16 dilution)
10	1-168	1 mg/ml	1 µg/ml
13	76-209	2.5 mg/ml	5 µg/ml
14	76-168	2 mg/ml	-
15	105-209	5 mg/ml	5-10µg/ml
17	105-168	250µg/ml	-
19	137-204	3.6 mg/ml	3.5 µg/ml
20	137-180	250 µg/ml	16 µg/ml

The truncated proteins deemed to be the most important in characterizing the hypersensitive response elicitor were chosen for large scale expression. The positive control (full length hypersensitive response elicitor) was expressed at a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also; however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The



negative control contained several background proteins as expected, but no obviously induced dominant protein.

### **Example 12 - Induction of HR in Tobacco**

5

The full length positive control protein elicited HR down to only 5-7µg/ml. The negative control (pET 28) imidazole purified “protein” - which contained only background proteins - elicited an HR response down to the 1:2 dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could not be used. This false HR was likely due to an affinity of the imidazole used in the purification process to bind to one or several of the background proteins, thereby not completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false HR response.

10 One definitive domain encompassing a small internal region of the protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the smallest HR domain. The other potential HR domain is thought to be located in the N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It was difficult to confirm or narrow down the N-terminus HR domain due to the difficulties encountered in purifying these fragment proteins. The N-terminus fragment proteins had to be purified with urea as no protein was recovered when the native purification process was used. Consequently, these proteins precipitated during the renaturing process and were difficult or nearly impossible to get back into solution, thereby making it hard to run the proteins through the HR assay, as only soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain was only compounded by the fact that the negative control elicited false HR at the low dilution levels thereby reducing the sensitivity of the assay.

20 Surprisingly, when the internal HR domain was cleaved between a.a. 168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23) should not be attributed to the internal HR domain, but rather to some other domain, leading to the assumption that there was likely a second HR domain to be found in the N-terminal region of the protein. However, as discussed earlier it was difficult to confirm this assumption.

30

The hypersensitive response elicitor C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, cleaving the protein between amino acids 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit HR. It was not expected that there would be an HR from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR.

### **Example 13 – Induction of Plant Growth Enhancement (PGE)**

The C-terminal fragments enhanced the growth of tomato by 9% to 21%. The N-terminal fragments enhanced the growth of tomato by 4% to 13%. The internal fragments enhanced growth by 9% to 20%. The 76-209 fragment enhanced growth by 18% at a concentration of 60 µg/ml, but not at the typical 20 µg/ml. This was attributed to the inaccuracy of the quantification process (Table 4).

Table 4

Fragment #	Amino acids	PGE ht>buffer @ 10 µg/ml	PGE ht>buffer @ 20 µg/ml
1 (+ control)	1-403	12%	11%
2 (- control)	-	-3%	-2%
4	169-403	9%	12%
5	210-403	13%	14%
			16% @ 40µg/ml
6	267-403	21%	21%
			23% @ 40µg/ml
7	343-403	7%	7%
9	1-104	4%	8%
10	1-168	13%	5%
13	76-209	7%	4%
			18% @ 60µg/ml
14	76-168	18%	20%
15	105-209	14%	19%
17	105-168	19%	16%
19	137-204	11%	13%
20	137-180	--	9%

\*A height greater than 10% above the buffer control was necessary to pass the PGE assay.

The oligopeptides enhanced growth from 7.4% to 17.3% (Table 5).

Table 5

Fragment	Amino acids	Expression	HR titer	TMV efficacy	PGE ht>buffer
oligo	150-179	NA	-	72.9%	10.1%
oligo	137-166	NA	-	61.2%	12.0%
oligo	121-150	NA	-	60.0%	17.3%
oligo	137-156	NA	-	-87.7%	7.4%

The data suggests that there is more than one PGE domain, although the C-terminal and internal domains appear to be dominant over the N-terminal domain, as the N-terminal fragments enhanced growth the least amount.

#### **Example 14 – Induction of Systemic Acquired Resistance (SAR)**

All of the hypersensitive response elicitor fragments tested to date appear to have 60% efficacy or greater, except for the oligopeptide 137-156 (Tables 5 and 6).

Table 6

Fragment #	Amino acids	Efficacy of TMV control
1 (+ control)	1-403	84% & 72%
2 (- control)	-	40% & 31%
4	169-403	64% & 79%
5	210-403	77% and 78%
6	267-403	70% and 72%
9	1-104	82%
10	1-168	69%
13	76-209	44% and 84%
14	76-168	83% & 87%
15	105-209	57% and 67%
17	105-168	89%
19	137-204	89% & 77%
20	137-180	64% & 58%

These data suggest that there are multiple SAR domains within the protein.

#### **Example 15 – Relationship Between HR, PGE, and SAR**

It is clear that the hypersensitive response activity is separable from the plant growth enhancement activity. The C-terminal fragments clearly enhance the growth of tomato by ca. 20% at a concentration of only 20 µg/ml, but these same fragments were not able to elicit HR in tobacco, even at higher concentrations than 200 µg/ml. The SAR activity also appears to be separable from the HR activity. This finding is highly significant for future work on transgenic applications of the hypersensitive response elicitor technology. The fragments that induce PGE and/or SAR but do not elicit HR will be imperative for this technology, as constitutive expression of even low levels of an HR elicitor might kill a plant.

#### **Example 16 - Non-HR Eliciting Fragments Derived from the Hypersensitive Response Elicitor from *Pseudomonas syringae* pv. *syringae* Induce Resistance in Tobacco to TMV and Promote the Growth of Tomato**

To test whether non-HR eliciting fragments derived from HrpZ, the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, is able to induce disease resistance, several fragment constructs were made and the expressed

fragment proteins were tested for HR elicitation and disease resistance induction in tobacco and growth promotion in tomato.

The following segments of *hrpZ*, the gene encoding the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, were amplified by PCR using Pfu Turbo (Stratagene): Regions coding for amino acids 152-190, aa 152-294, aa 190-294, aa 301-341, and full length HrpZ (aa 1-341). The DNA fragments were cloned into pCAL-n (Stratagene) to create C-terminal fusion proteins to the calmodulin-binding peptide. pCAL-n was chosen, because the fusion protein could be easily and gently purified on calmodulin resin. The DNA was transformed into *E. coli* DH5 $\alpha$ , and the correct clones were identified. The clones were then transferred to *E. coli* BLR DE3 for protein expression. The bacteria were grown in Terrific Broth to an OD<sub>620</sub> of 0.8-1.0. Protein expression was then induced with IPTG and the bacteria were incubated for an additional 3 h. All of the HrpZ fragments were able to be expressed this way.

Amino acid fragments 152-294 and 190-294 were chosen for further analysis and characterization. It was expected that the fragment 152-294 contained a domain that elicited the HR, while fragment 190-294 contained no domain that elicited the HR. The cultures were spun down, and the bacteria resuspended in 40 ml of 10 mM Tris pH 8.0. Twenty  $\mu$ l of antifoam and 40  $\mu$ l of 200 mM PMSF were added, and the bacteria was sonicated to break open the cells. The bacterial debris was removed by centrifugation, and the supernatant was placed in a boiling water bath for 10 min. The precipitate was removed by centrifugation and the supernatant, a crude protein preparation, was retained for tests.

Fifteen  $\mu$ l of each supernatant was run on a gel and stained to determine if the protein was present. It was estimated that about five times as much of the 152-294 fragment was present as the 190-294 fragment. Several dilutions of each preparation were infiltrated into tobacco leaves on two plants for HR tests (Table 7). As shown in Table 7, the 152-294 fragment elicited an HR, but the 190-294 fragment did not.

Table 7

HR test results of HrpZ fragments

<u>HrpZ Fragment</u>	<u>Dilution of Fragment Preparation<sup>a</sup></u>			
	<u>1:2</u>	<u>1:5</u>	<u>1:25</u>	<u>1:125</u>
152-294	+,+ <sup>b</sup>	+,+	+,+	-, -
190-294	-,-	-,-	-,-	-,-

<sup>a</sup>The preparations were diluted with MilliQ water.

<sup>b</sup>The results are indicated for each of two plants. +, HR; -, no HR.

10

The fragment preparations were then tested for inducing resistance to TMV and for growth enhancement. Due to the difference in concentration of the HrpZ fragments, the 152-294 preparation was diluted 40-fold and the 190-294 preparation was diluted 8-fold. The results showed that the 190-294 aa fragment reduced the number of TMV lesions by 85% in comparison to buffer controls (Table 8). In contrast, the 152-294 aa fragment reduced the number of TMV lesions by only 55%. As also shown in Table 8, plants treated with the 152-294 aa fragment grew 4.64% more than buffer treated plants, while plants treated with the 190-294 aa fragment grew 2.62% more than the buffer treated plants.

Table 8

HR test, TMV, and PGE test results

<u>HrpZ Fragment</u>	<u>HR elicitation<sup>a</sup></u>	<u>TMV (% efficacy)<sup>b</sup></u>	<u>PGE(% &gt; buffer ht)<sup>c</sup></u>
152-294	+	54.64	4.64
190-294	-	85.25	2.62

<sup>a</sup>+, elicits HR in tobacco leaves; -, no HR in tobacco leaves.

<sup>b</sup>% reduction in TMV lesions in unsprayed leaf of tobacco.

<sup>c</sup>% greater height than buffer sprayed plants.

30

The results of these tests show that amino acids 152-190 appear to be involved in HR elicitation, because their removal eliminated the ability to elicit the HR. Both fragment preparations achieved disease control and growth enhancement. Thus, the ability to elicit the HR is not the determining factor for reduction in TMV infection and growth enhancement.

35

**Example 17 - Use of 13 Amino Acid Peptide Derived from *Phytophthora megasperma* Stimulates Tomato Seedling Growth**

Parsley leaves develop a typical resistance reaction against the soybean pathogen *Phytophthora megasperma* comprising hypersensitive cell death, defense related gene activation, and phytoalexin formulation. Several years ago, a 42 kDa glycoprotein elicitor was purified from the fungal culture filtrate of *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f.sp. glycinea Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991), which is hereby incorporated by reference). Then, an oligopeptide of 13 amino acid was identified within the 42 kDa glycoprotein. The 13 amino acids peptide appeared to have similar biological activity as that of the full length glycoprotein (42 kDa). It is sufficient to elicit a complex defense response in parsley cells including H<sup>+</sup>/Ca<sup>2+</sup> influxes, K<sup>+</sup>/Cl<sup>-</sup> effluxes, active oxygen production, SAR gene induction, and phytoalexin compound accumulation (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Response," Cell 78:449-460 (1994), which is hereby incorporated by reference).

To test if the 13 amino acid peptide derived from the 42 kDa protein also enhanced plant growth, 20 mg of the oligopeptide was synthesized from Biosynthesis Corp. The synthesized sequence of the peptide is NH<sub>2</sub>-Val-Trp-Asn-Gln-Pro-Val-Arg-Gly-Phe-Lys-Val-Tyr-Glu-COOH (SEQ. ID. No. 39). The synthesized peptide was resuspended in 10 ml of 5 mM potassium phosphate buffer and, then, diluted to 1 and 100 ng/ml with the same buffer. About 100 tomato seeds (variety, Marglobe) were submerged in 20 ml of peptide solution overnight. The soaked seeds were planted in an 8 inch pot with artificial soil. Seeds soaked in the buffer without the peptide were used as a control. After seedlings emerged and the first two true leaves fully expanded, the height of the tomato seedlings was recorded. The peptide was not able to elicit the HR in tobacco and other tested plants. However, it had a profound effect on plant growth promotion. Table 9 shows that tomato seedlings treated with the peptide increased 12.6 % in height, indicating that the fungal peptide derived from the 42 kDa glycoprotein can promote tomato seedling growth. Extended studies showed that the peptide also had similar growth

5

10

15

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.



**WHAT IS CLAIMED:**

1. An isolated fragment of a hypersensitive response elicitor protein or polypeptide, wherein said fragment does not elicit a hypersensitive response but has other activity in plants.
2. An isolated fragment according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas*, *Xanthomonas*, or *Phytophthora*.
3. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.
4. An isolated fragment according to claim 3, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.
5. An isolated fragment according to claim 4, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.
6. An isolated fragment according to claim 4, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.
7. An isolated fragment according to claim 4, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

8. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

9. An isolated fragment according to claim 8, wherein the  
5 fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

10. An isolated DNA molecule encoding a fragment according to claim 1.

10 11. An isolated DNA molecule according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas*, *Xanthomonas*, or *Phytophthora*.

12. An isolated DNA molecule according to claim 11, wherein the  
15 hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

13. An isolated DNA molecule according to claim 12, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino  
20 acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

14. An isolated DNA molecule according to claim 12, wherein the  
25 fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.

15. An isolated DNA molecule according to claim 12, wherein the  
30 fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.

16. An isolated DNA molecule according to claim 12, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

5

17. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

18. An isolated DNA molecule according to claim 18, wherein the  
10 fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

19. An expression system transformed with a DNA molecule according to claim 10.

20. An expression system according to claim 19, wherein said  
15 DNA molecule is in proper sense orientation and correct reading frame.

21. A host cell transformed with a DNA molecule according to  
claim 10.

20

22. A host cell according to claim 21, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

23. A host cell according to claim 21, wherein the DNA molecule  
25 is transformed with an expression system.

24. A transgenic plant transformed with the DNA molecule of claim 10.

25. A transgenic plant according to claim 24, wherein the plant is  
30 selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive,

cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5

26. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

10

27. A transgenic plant seed transformed with the DNA molecule of claim 10.

15

28. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

29. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25

30. A method of imparting disease resistance to plants comprising:  
applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.

30

31. A method according to claim 30, wherein plants are treated during said applying.

32. A method according to claim 30 wherein plant seeds are treated during said applying, said method further comprising:

5                   planting the seeds treated with the fragment of the  
hypersensitive response elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

33. A method of enhancing plant growth comprising:

10                   applying a fragment of a hypersensitive response elicitor  
protein or polypeptide, which fragment does not elicit a hypersensitive response, in a  
non-infectious form to a plant or plant seed under conditions effective to enhance  
plant growth.

15                   34. A method according to claim 33, wherein plants are treated  
during said applying.

35. A method according to claim 33, wherein plant seeds are treated during said applying, said method further comprising:

20                   planting the seeds treated with the fragment of the  
hypersensitive response elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

36. A method of insect control for plants comprising:

25                   applying a fragment of a hypersensitive response elicitor protein or  
polypeptide, which fragment does not elicit a hypersensitive response, in a non-  
infectious form to a plant or plant seed under conditions effective to control insects.

37. A method according to claim 36, wherein plants are treated during said applying.

30

38. A method according to claim 36, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the fragment of the  
hypersensitive response elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

5                    39.    A method of imparting disease resistance to plants comprising:  
                         providing a transgenic plant or plant seed transformed with a  
                         DNA molecule which encodes a fragment of a hypersensitive response elicitor protein  
                         or polypeptide, which fragment does not elicit a hypersensitive response, and  
                         growing the transgenic plant or transgenic plants produced  
10                    from the transgenic plant seeds under conditions effective to impart disease resistance.

                         40.    A method according to claim 39, wherein a transgenic plant is  
                         provided.

15                    41.    A method according to claim 39, wherein a transgenic plant  
                         seed is provided.

                         42.    A method of enhancing plant growth comprising:  
                         providing a transgenic plant or a plant seed transformed with a  
20                    DNA molecule which encodes a fragment of a hypersensitive response elicitor protein  
                         or polypeptide, which fragment does not elicit a hypersensitive response, and  
                         growing the transgenic plant or transgenic plants produced  
                         from the transgenic plant seeds under conditions effective to enhance plant growth.

25                    43.    A method according to claim 42, wherein a transgenic plant is  
                         provided.

                         44.    A method according to claim 42, wherein a transgenic plant  
                         seed is provided.

30                    45.    A method of insect control for plants comprising:

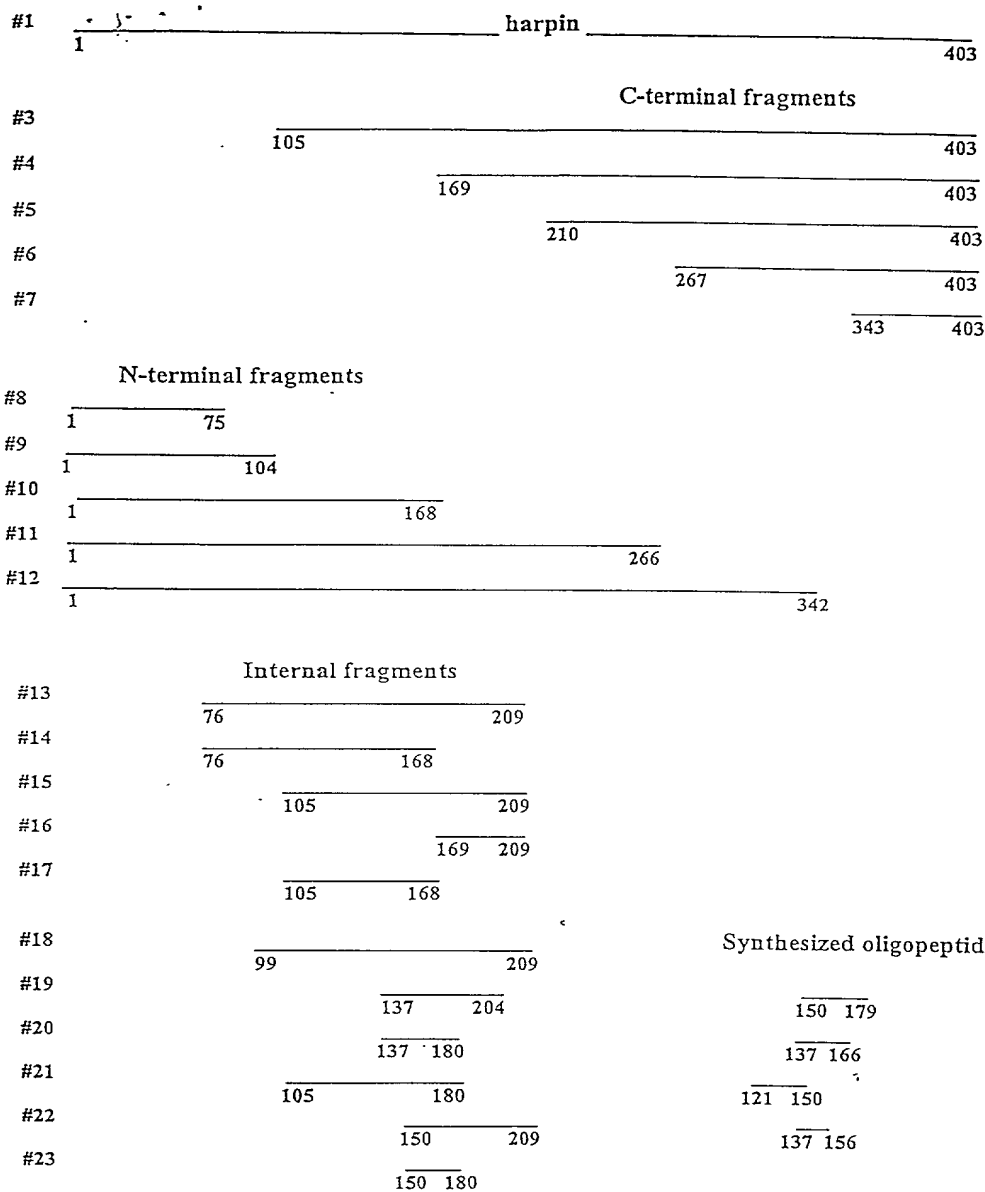


### ABSTRACT OF THE DISCLOSURE

The present invention is directed to isolated active fragments of a hypersensitive response elicitor protein or polypeptide which fragment does not elicit a hypersensitive response in plants. Also disclosed are isolated DNA molecules which encode such fragments. Isolated fragments of hypersensitive response elicitor proteins or polypeptides in accordance with the present invention and the isolated DNA molecules that encode them have the following activities: imparting disease resistance to plants, enhancing plant growth, and/or controlling insects on plants. This can be achieved by applying the fragments of a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the fragment can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

03442400 10049





Harpin fragments derived from HrpN of *Erwinia amylovora*

FIGURE 1

1; 5'-GGGAATTCATATGAGTCTGAATACAAGTGGG-3'  
 76; 5'-GGGAATTCATATGGGCGGTGGCTTAGGCGGT-3'  
 99; 5'-GGCATATGTCTGAACGCGCTGAACGATATG-3'  
 105; 5'-GGGAATTCATATGTTAGGCGGTTCGCTGAAC-3'  
 110; 5'-GGCATATGCTGAACACGCTGGGCTCGAAA-3'  
 137; 5'-GGCATATGTCAACGTCCCAAAACGACGAT-3'  
 150; 5'-GGCATATGTCCACCTCAGACTCCAGCG-3'  
 169; 5'-GGGAATTCATATGCAAAGCCTGTTTGGTGATGGG-3'  
 170; 5'-GGGAATTCATATGGGTAATGGTCTGAGCAAG-3'  
 176; 5'-GGGAATTCATATGAAAGCGGGCATTGAGGCG-3'  
 184; 5'-GGGAATTCATATGACACCAGCCAGTATGGAGCAG-3'  
 195; 5'-GCAAGCTTAAACAGCCCACCACCGCCCATCAT-3'  
 204; 5'-GCAAGCTTAAATCGTTCAGCGCGTTCGACAG-3'  
 216; 5'-GCAAGCTTAAATATCTCGCTGAACATCTTCAGCAG-3'  
 218; 5'-GCAAGCTTAAAGGTGCCATCTTGCCCATCAC-3'  
 220; 5'-GCAAGCTTAAATCAGTGACTCCTTTTTTATAGGC-3'  
 229; 5'-GCAAGCTTAAACAGGCCCCGACAGCGCATCAGT-3'  
 226; 5'-GCAAGCTTAAACCGATACCGGTACCCACGGC-3'  
 234; 5'-GCAAGCTTAAATCCGTCGTCATCTGGCTTGCTCAG-3'  
 240; 5'-GCAAGCTTAAAGCCGCGCCAGCTTG-3'

Oligonucleotide primers used for the construction of  
 the subclones of *Erwinia amylovora* hrpN.

FIGURE 2

COMBINED DECLARATION FOR PATENT  
APPLICATION AND POWER OF ATTORNEY  
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER  
21829/31 (EBC-002)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A  
HYPERSENSITIVE RESPONSE**

the specification of which (check only one item below):

☒ is attached hereto.

☐ was filed as U.S. Patent Application Serial No. \_\_\_\_\_ on \_\_\_\_\_ and was amended on \_\_\_\_\_  
(if applicable).

☐ was filed as PCT International Application Number \_\_\_\_\_ on \_\_\_\_\_ and was amended under PCT Article 19  
on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specifications, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

**PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:**

COUNTRY (IF PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
United States	60/103,050	05-OCT-1998	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
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			<input type="checkbox"/> YES <input type="checkbox"/> NO
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<b>COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Continued)</b> (Includes Reference to PCT International Applications)				<b>ATTORNEY'S DOCKET NUMBER</b> <b>21829/31 (EBC-002)</b>	
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT International filing date of this application:					
<b>PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:</b>					
U.S. APPLICATIONS			STATUS (Check One)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED	
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			
<b>POWER OF ATTORNEY:</b> As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. <b>Michael L. Goldman, Registration No. 30,727, Gunnar G. Leinberg, Registration No. 35,584; Dennis M. Connolly, Registration No. 40,964; Edwin V. Merkel, Registration No. 40,087, Jeffery B. Arnold, Registration No. 39,540, Georgia Caton, Registration No. P-44,597</b>					
Send Correspondence to: <b>Michael L. Goldman NIXON PEABODY LLP Clinton Square, P.O. Box 1051 Rochester, New York 14603</b>			Direct telephone calls to: <b>Michael L. Goldman (716) 263-1304</b>		
201	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE/FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	P.O. ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
		Wei	Zhong-Min		
202	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE/FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	P.O. ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
		Fan	Hao		
203	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE/FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	P.O. ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
		Bothell	Washington	China	
203	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE/FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	P.O. ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
		Niggemeyer	Jennifer	L.	
203	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE/FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	P.O. ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
		Bothell	Washington	United States	
		Bothell	Washington	Washington 98021/USA	
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.					
SIGNATURE OF INVENTOR 201		SIGNATURE OF INVENTOR 202		SIGNATURE OF INVENTOR 203	
UNSigned		UNSigned		UNSigned	
DATE		DATE		DATE	

# SEQUENCE LISTING

<110> Wei, Zhong-Min  
Fan, Hao  
Niggemeyer, Jennifer L.

<120> HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE  
ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

<130> 21829/31 (EBC-002)

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<151> 1998-10-05

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<213> *Erwinia amylovora*

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<212> DNA

<213> *Erwinia amylovora*

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<213> *Erwinia amylovora*

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<212> PRT

<213> *Erwinia amylovora*

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 <213> *Erwinia amylovora*

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caacaggctg	cggacagcca	cagcatggaa	ttaggatctg	ccagtcacag	cacgacctac	4980
aataatctgt	cgagaataaa	taatgacggc	attgtcgagc	tgctacacaa	acatttcgat	5040
gcggcattac	cagcaagcag	tgccaaacgt	cttgggtgaa	tgatgaataa	cgatccggca	5100
ctgaaagata	ttattaagca	gctgcaaagt	acgccgttca	gcagcgccag	cgtgtcgatg	5160



Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile  
 195 200 205  
 Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala  
 210 215 220  
 Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln  
 225 230 235 240  
 Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro  
 245 250 255  
 Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys  
 260 265 270  
 Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln  
 275 280 285  
 Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val  
 290 295 300  
 Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro  
 305 310 315 320  
 Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys  
 325 330 335  
 Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln  
 340 345 350  
 His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr  
 355 360 365  
 Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys  
 370 375 380  
 Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys  
 385 390 395 400  
 Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr  
 405 410 415  
 Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile  
 420 425 430  
 Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg  
 435 440 445

Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp  
 450 455 460

Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp  
 465 470 475 480

Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser  
 485 490 495

Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser  
 500 505 510

Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg  
 515 520 525

His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His  
 530 535 540

Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His  
 545 550 555 560

Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg  
 565 570 575

Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro  
 580 585 590

Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His  
 595 600 605

Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe  
 610 615 620

His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg  
 625 630 635 640

Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly  
 645 650 655

Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His  
 660 665 670

His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly  
 675 680 685

Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr  
 690 695 700

Lys	Gly	Trp	Thr	Gly	Ala	Glu	Ser	Asp	Cys	Lys	Gln	Leu	Lys	Lys	Gly	705	710	715	720
Leu	Asp	Gly	Ala	Ala	Tyr	Leu	Leu	Lys	Asp	Gly	Glu	Val	Lys	Arg	Leu	725	730	735	
Asn	Ile	Asn	Gln	Ser	Thr	Ser	Ser	Ile	Lys	His	Gly	Thr	Glu	Asn	Val	740	745	750	
Phe	Ser	Leu	Pro	His	Val	Arg	Asn	Lys	Pro	Glu	Pro	Gly	Asp	Ala	Leu	755	760	765	
Gln	Gly	Leu	Asn	Lys	Asp	Asp	Lys	Ala	Gln	Ala	Met	Ala	Val	Ile	Gly	770	775	780	
Val	Asn	Lys	Tyr	Leu	Ala	Leu	Thr	Glu	Lys	Gly	Asp	Ile	Arg	Ser	Phe	785	790	795	800
Gln	Ile	Lys	Pro	Gly	Thr	Gln	Gln	Leu	Glu	Arg	Pro	Ala	Gln	Thr	Leu	805	810	815	
Ser	Arg	Glu	Gly	Ile	Ser	Gly	Glu	Leu	Lys	Asp	Ile	His	Val	Asp	His	820	825	830	
Lys	Gln	Asn	Leu	Tyr	Ala	Leu	Thr	His	Glu	Gly	Glu	Val	Phe	His	Gln	835	840	845	
Pro	Arg	Glu	Ala	Trp	Gln	Asn	Gly	Ala	Glu	Ser	Ser	Ser	Trp	His	Lys	850	855	860	
Leu	Ala	Leu	Pro	Gln	Ser	Glu	Ser	Lys	Leu	Lys	Ser	Leu	Asp	Met	Ser	865	870	875	880
His	Glu	His	Lys	Pro	Ile	Ala	Thr	Phe	Glu	Asp	Gly	Ser	Gln	His	Gln	885	890	895	
Leu	Lys	Ala	Gly	Gly	Trp	His	Ala	Tyr	Ala	Ala	Pro	Glu	Arg	Gly	Pro	900	905	910	
Leu	Ala	Val	Gly	Thr	Ser	Gly	Ser	Gln	Thr	Val	Phe	Asn	Arg	Leu	Met	915	920	925	
Gln	Gly	Val	Lys	Gly	Lys	Val	Ile	Pro	Gly	Ser	Gly	Leu	Thr	Val	Lys	930	935	940	
Leu	Ser	Ala	Gln	Thr	Gly	Gly	Met	Thr	Gly	Ala	Glu	Gly	Arg	Lys	Val	945	950	955	960

Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr  
 965 970 975  
 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His  
 980 985 990  
 Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly  
 995 1000 1005  
 Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro  
 1010 1015 1020  
 Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His  
 1025 1030 1035 1040  
 Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu  
 1045 1050 1055  
 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val  
 1060 1065 1070  
 Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly  
 1075 1080 1085  
 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu  
 1090 1095 1100  
 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu  
 1105 1110 1115 1120  
 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp  
 1125 1130 1135  
 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro  
 1140 1145 1150  
 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val  
 1155 1160 1165  
 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser  
 1170 1175 1180  
 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe  
 1185 1190 1195 1200  
 Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr  
 1205 1210 1215

Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp  
 1220 1225 1230  
 Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val  
 1235 1240 1245  
 Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu  
 1250 1255 1260  
 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser  
 1265 1270 1275 1280  
 Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val  
 1285 1290 1295  
 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly  
 1300 1305 1310  
 Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly  
 1315 1320 1325  
 Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile  
 1330 1335 1340  
 Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys  
 1345 1350 1355 1360  
 Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile  
 1365 1370 1375  
 Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly  
 1380 1385 1390  
 Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro  
 1395 1400 1405  
 Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu  
 1410 1415 1420  
 Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr  
 1425 1430 1435 1440  
 Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn  
 1445 1450 1455  
 Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser  
 1460 1465 1470

Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg	1475	1480	1485
Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn	1490	1495	1500
Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala	1505	1510	1515 1520
Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly	1525	1530	1535
Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu	1540	1545	1550
Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu	1555	1560	1565
Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys	1570	1575	1580
His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu	1585	1590	1595 1600
Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His	1605	1610	1615
Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg	1620	1625	1630
Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser	1635	1640	1645
Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser	1650	1655	1660
Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp	1665	1670	1675 1680
Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn	1685	1690	1695
Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro	1700	1705	1710
Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu	1715	1720	1725



Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val  
 1730 1735 1740

Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser  
 1745 1750 1755 1760

Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu  
 1765 1770 1775

Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile  
 1780 1785 1790

Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg  
 1795 1800 1805

Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser  
 1810 1815 1820

Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser  
 1825 1830 1835

<210> 29  
 <211> 420  
 <212> DNA  
 <213> *Erwinia amylovora*

<400> 29  
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 acgcccatac atctgaaaga cggggtgtgc gccctgtata acgaacaaga tgaggaggcg 120  
 gcggtgctgg aagtaccgca acacagcgac agcctgttac tacactgccg aatcattgag 180  
 gctgaccac aaacttcaat aaccctgtat tcgatgctat tacagctgaa ttttgaaatg 240  
 gcggccatgc gcggctgttg gctggcgctg gatgaactgc acaacgtgcg tttatgtttt 300  
 cagcagtcgc tggagcatct ggatgaagca agtttttagcg atatcgtag cggcttcac 360  
 gaacatgcgg cagaagtgcg tgagtatata gcgcaattag acgagagtag cgcggcataa 420

<210> 30  
 <211> 139  
 <212> PRT  
 <213> *Erwinia amylovora*

<400> 30  
 Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser  
 1 5 10 15

Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu  
 20 25 30

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His  
35 40 45

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln  
50 55 60

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met  
65 70 75 80

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val  
85 90 95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe  
100 105 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu  
115 120 125

Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala  
130 135

<210> 31

<211> 341

<212> PRT

<213> Pseudomonas syringae

<400> 31

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
1 5 10 15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
20 25 30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
65 70 75 80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met



<210> 32  
 <211> 1026  
 <212> DNA  
 <213> *Pseudomonas syringae*

<400> 32  
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 gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc 120  
 gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga 180  
 aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc 240  
 atcgtgctgc tggacaagct gatccatgaa aagctcgggtg acaacttcgg cgcgtctgctg 300  
 gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc 360  
 aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac 420  
 gatatgccga tgctgaacaa gatcgcgcag ttcatggatg acaatccgc acagtttccc 480  
 aagccggact cgggtcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac 540  
 gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag 600  
 agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc 660  
 aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc 720  
 ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780  
 tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgagac cggtagctcg 840  
 gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag 900  
 ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960  
 gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca 1020  
 gcctga 1026

<210> 33  
 <211> 1729  
 <212> DNA  
 <213> *Pseudomonas syringae*

<400> 33  
 tccacttcgc tgattttgaa attggcagat tcatagaaac gttcaggtgt ggaaatcagg 60  
 ctgagtgcgc agatttcgtt gataaggggtg tggtagtggc cattgttggc catttcaagg 120  
 cctctgagtg cgggtgcggag caataaccagt cttcctgctg gcgtgtgcac actgagtcgc 180  
 aggcataaggc atttcagttc cttgcgttgg ttgggcatat aaaaaaagga acttttaaaa 240  
 acagtgcaat gagatgccgg caaaacggga accggtcgct gcgctttgcc actcacttcg 300  
 agcaagctca accccaaaca tccacatccc tatcgaacgg acagcgatac ggccacttgc 360  
 tctggtaaac cctggagctg gcgtcgggtcc aattgccac ttagcgaggt aacgcagcat 420  
 gagcatcggc atcacacccc ggccgcaaca gaccaccag ccactcgatt tttcggcgct 480  
 aagcggcaag agtcctcaac caaacacggt cggcgagcag aacactcagc aagcgatcga 540  
 cccgagtgca ctgttggtcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600  
 cagcaccgtc cagaatccgc aggaagccag caagcccaac gacagccagt ccaacatcgc 660  
 taaattgatc agtgcatcga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa 720  
 gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct 780  
 cggtagaccg tcggccgata gcggggggcg cggtagaccg gatgcgacag gtggcggcgg 840  
 cggtgatacg ccaagcgcaa caggcggtgg cggcggtgat actccgaccg caacaggcgg 900  
 tggcggcagc ggtggcggcg gcacaccac tgcaacaggt ggcggcagcg gtggcacacc 960  
 cactgcaaca ggcggtggcg aggggtggcgt aacaccgcaa atcactccgc agttggccaa 1020

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ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080
cggcaagatc aatgtgggtga aagacacccat caaggctcggc gctggcgaag tctttgacgg 1140
ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260
cgaggtcgat ggcattccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320
gcatgcccag aacgtcggtg aagacctgat tacggtcaaa ggcgaggag gcgcagcggc 1380
cactaatctg aacatcaaga acagcagtg caaagggtgca gacgacaagg ttgtccagct 1440
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt 1500
tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc 1560
taaccacggc aagttcgccc tggtgaaaag cgacagtgac gatctgaagc tggcaacggg 1620
caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgaccaaca 1680
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc 1729

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<210> 34

<211> 424

<212> PRT

<213> *Pseudomonas syringae*

<400> 34

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Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
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Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
      20                      25                      30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
      35                      40                      45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
      50                      55                      60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
      65                      70                      75                      80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
      85                      90                      95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
      100                      105                      110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
      115                      120                      125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
      130                      135                      140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
      145                      150                      155                      160

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Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly  
 165 170 175  
 Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr  
 180 185 190  
 Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr  
 195 200 205  
 Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile  
 210 215 220  
 Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp  
 225 230 235 240  
 Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp  
 245 250 255  
 Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr  
 260 265 270  
 Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val  
 275 280 285  
 Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln  
 290 295 300  
 Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala  
 305 310 315 320  
 Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp  
 325 330 335  
 Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe  
 340 345 350  
 Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln  
 355 360 365  
 Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly  
 370 375 380  
 Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr  
 385 390 395 400  
 Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln  
 405 410 415

Ala Ser Thr Gln His Thr Glu Leu  
420

<210> 35

<211> 344

<212> PRT

<213> Pseudomonas solanacearum

<220>

<223> Description of Unknown Organism: Pseudomonas  
solanacearum

<400> 35

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln  
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Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser  
20 25 30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile  
35 40 45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly  
50 55 60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala  
65 70 75 80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser  
85 90 95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met  
100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190  
 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
 195 200 205  
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220  
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240  
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255  
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270  
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285  
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300  
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320  
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335  
 Gln Ser Thr Ser Thr Gln Pro Met  
 340

<210> 36

<211> 1035

<212> DNA

<213> *Pseudomonas solanacearum*

<400> 36

atgtcagtcg gaaacatcca gagcccgtcg aacctcccgg gtctgcagaa cctgaacctc 60  
 aacaccaaca ccaacagcca gcaatcgggc cagtcctgtc aagacctgat caagcaggtc 120  
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<212> PRT

<213> *Xanthomonas campestris* pv. *glycines*

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<211> 20

<212> PRT

<213> *Xanthomonas campestris* pv. *pelargonii*

<400> 38

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<210> 39

<211> 13

<212> PRT

<213> *Phytophthora megasperma*

<400> 39

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